

SPECIFIC OXIDATION OF RETINOIC ACID TO 4-OXO-RETINOIC ACID IN DILUTED ACID SOLUTIONS

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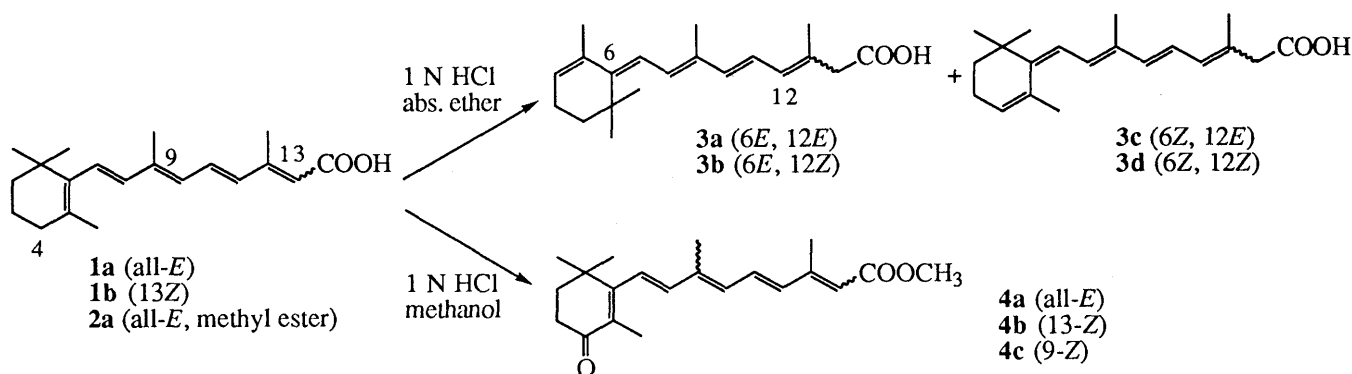
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Treatment of retinoic acid (**1a**) or its methyl ester (**2a**) with hydrochloric acid in methanol gave methyl 4-oxo-retinoates (**4a - c**). Similar oxidation proceeded when **2a** was treated with trifluoromethanesulfonic acid in the presence of lithium chloride in methanol.

KEY WORDS vitamin A; retinoic acid; allylic oxidation; trifluoromethanesulfonic acid

Retinoic acid (vitamin A acid, **1a**, all-*E*) has recently attracted much attention because of its key roles in cell differentiation and proliferation,¹⁾ and in morphogenesis of vertebrates.²⁾ Biological investigations have shown that retinoic acid (**1a**) elicits various specific activities by the direct control of gene expressions through binding to the specific nuclear receptor(s).^{1,3)} Therefore, retinoic acid (**1a**) should be classified as a "hormone", similar to the steroid hormones, thyroid hormones, and activated vitamin D₃, although the precise mechanisms of its actions are still unclear. Recently, several metabolites of **1a**, such as 9-*cis*-retinoic acid⁴⁾ and 4-oxo-retinoic acid,⁵⁾ have been proven to participate in or to exhibit specific activities of retinoic acid (**1a**). Structurally, retinoic acid (**1a**) consists of a conjugated pentaene system which seems to be very prone to isomerization or oxidation, either enzymatically or chemically. An understanding of the fundamental chemical behaviors of retinoic acid (**1a**) is important for biological investigations on retinoic acid (**1a**) and its metabolites, and for clinical applications in the fields of dermatology and oncology.⁶⁾ Previously, we reported that retinoic acid (**1a**) easily decomposed in various acidic conditions to give a mixture of cyclized isomers.⁷⁾ The reactions strongly depended on the acidity of the solution. In the course of our investigations on the acid-catalyzed isomerization of **1a**, we found an unusual, specific oxidation of **1a** at the 4 position. In this paper, the allylic oxidations of **1a** and its methyl ester (**2a**) in dilute acids are discussed, in comparison with the *retro*-isomer formation.

Treatment of **1a** with gaseous hydrogen chloride (1 M solution, 60 eq to **1a**) in anhydrous ether at 18°C for 24 h gave 4,14-*retro*-retinoic acids (**3a - d**, 12 %), which consist of four geometrical isomers at the 6 and 12 positions, besides the recovered **1a** (30 %) and its 13*Z*-isomer (**1b**, 17 %). In this reaction condition, no cyclized compound, such as can be formed by treating **1a** with strong acid,⁷⁾ was not detected. The structures of **3a - d** were determined from ¹H-NMR chemical shifts,^{8,9)} after esterification with CH₂N₂. On the other hand, when **1a** was treated with hydrochloric acid in methanol (1 M solution, 60 equiv to **1a**) at 18°C for 24 h under argon,¹⁰⁾ the *retro*-isomers were not formed and three other neutral products **4a - c** were obtained in 24 %, 5 % and 16 % yields, respectively. The major product (**4a**, pale yellow prisms from *n*-hexane, mp 94.5 -



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Table I. ^1H NMR Chemical Shifts^{a)} and NOE of Methyl 4-*oxo*-Retinoates **4a** - **c**

	H ₂	H ₃	H ₇	H ₈	H ₁₀	H ₁₁	H ₁₂	H ₁₄	H _{16,17}	H ₁₈	H ₁₉	H ₂₀	OCH ₃
4a	1.86	2.51	6.33	6.33	6.26	6.99	6.37	5.82	1.19	1.86	2.04	2.36	3.72
4b	1.86	2.51	6.31	6.36	6.37	6.96	7.84	5.70	1.19	1.85	2.02	2.08	3.71
4c	1.88	2.53	6.33	6.84	6.20	7.01	6.28	5.80	1.20	1.89	2.03	2.32	3.72

^{a)} Chemical shifts are expressed in ppm relative to Me₄Si in CDCl₃.

95°C) has the molecular formula C₂₁H₂₈O₃, with two carbonyl groups (IR: 1665 cm⁻¹ due to a cyclic conjugated ketone and 1714 cm⁻¹ due to an ester) in the structure. From the ^1H -NMR spectrum, **4a** has no protons at the C₄ position and the signal of the H₃ protons is shifted to lower field (2.51 ppm) than that of **1a** (2.03 ppm). Therefore, **4a** is methyl 4-*oxo*-retinoate and the stereochemistry of the side chain was determined as all-*E* form from the chemical shifts (Table I) and NOE experiments.¹¹⁾ Two minor compounds, **4b** and **4c**, were isolated by HPLC (ODS; eluent: 20% H₂O-CH₃CN) and are also derivatives of methyl 4-*oxo*-retinoate. Comparisons of ^1H -NMR chemical shifts (Table I) and NOE experiments indicated that **4b** and **4c** are the 13*Z*- and 9*Z*-isomer of **4a**, respectively. This non-enzymatic oxidation is important, because 4-*oxo*-retinoic acid is believed to be one of the major metabolites of **1a**.

For eliminating the esterification process, the reactions of methyl retinoate (**2a**) were examined (Table II). The selective oxidation of **2a** at the 4 position proceeded under the same condition as for **1a** (entry #1). In HCl-anhydrous ether, methyl 4,14-*retro*-retinoate (**5a** - **d**) with **2a** and methyl 13-*cis*-retinoate (**2b**) were obtained, but the 4-*oxo* compounds were not detected. When methanol (10 % to ether) was added after 24 h to the reaction mixture (#2), containing **2** and **5**, methyl 4-*oxo*-retinoates (**4**) were formed in 29 % yield (#3). Treatment of **2a** with trifluoromethanesulfonic acid (TFSA, 80 eq) in methanol gave only methyl 4,14-*retro*-retinoates (**5**) in 24 % yield (#4). Interestingly, the addition of LiCl (60 eq) caused a dramatic change in the reaction, and methyl 4-*oxo*-retinoates (**4**) were obtained in 52 % yield (#5). Addition of KBr (60 eq) also oxidized **2a**, although the reaction was slower than #5, probably due to the low solubility of KBr in methanol. From these observations, the formation of methyl 4-*oxo*-retinoates (**4**) from **2a** seems to be influenced by three factors, acid, halide, and solvent. Using trifluoroacetic acid (TFA), which is a much weaker acid than TFSA or HCl, 93% of **2a** (with the isomer **2b**) was recovered after 24 h at 23°C.¹²⁾ This result indicates that the 4-*oxo* formation requires sufficient acidity to isomerize **2a** to the methyl 4,14-*retro*-retinoate (**5**).

Table II. Reaction of Methyl Retinoate (**2a**) under Various Acidic Conditions (23°C)

	Acid	Additive	Solvent	Time	Yield ^{a)}		
					2	4 (4- <i>oxo</i>)	5 (4,14- <i>retro</i>)
#1	1 N HCl (60 eq)	none	methanol	24 h	4 %	49 %	-
#2	1 N HCl (60 eq)	none	abs. ether	24 h	47 %	-	24 %
#3	1 N HCl (60 eq)	methanol ^{b)}	abs. ether	46 h	15 %	29 %	9 %
#4	TFSA (80 eq)	none	methanol	24 h	21 %	-	24 %
#5	TFSA (60 eq)	LiCl (60 eq)	methanol	24 h	9 %	52 %	-

^{a)} Yields were determined from ^1H -NMR integrations and are the sum of geometrical isomers.

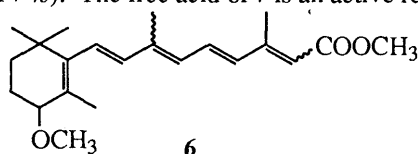
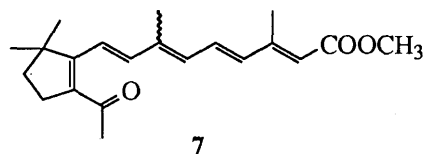
^{b)} Methanol (10 % to abs. ether) was added to the reaction mixture of **2a** in abs. ether after 24 h.

Several oxidations of retinoic acid (**1a**) or its derivatives have been reported.^{1,11)} In the present oxidation reaction of retinoic acid, a radical scavenger, such as hydroquinone or 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH), decreased the yield of 4-*oxo* derivatives, and oxygen also inhibited the reaction. Therefore, the oxidation is considered to proceed through a radical pathway, although the detailed mechanism is unclear. The reactivity of several conjugated unsaturated esters in TFSA-LiCl at room temperature was examined. Methyl 1-cyclohexenylcarboxylate and methyl 3-(2,6,6-trimethylcyclohexen-1-yl)propenoate did not react under this condition. Methyl 3-methyl-5-(2,6,6-trimethylcyclohexen-1-yl)pentadienoate afforded only the *retro*-isomers even in the presence of LiCl. β -Ionone, a synthon for vitamin A or carotenoids, was poorly oxidized at the 4 position with TFSA (60 equiv)-LiCl (60 eq), although the reaction afforded a complex mixture of compounds containing many acid-catalyzed aldol condensation products.¹³⁾ Thus, the oxidation by HCl-CH₃OH or TFSA-LiCl-CH₃OH seems to be very specific to retinoic acids or *retro*-retinoic acids.

In conclusion, retinoic acid is oxidized in diluted methanolic hydrochloric acid or in a similar acidic condition (methanolic TFSA-LiCl) to 4-*oxo* derivatives (**4**) in moderate yields. Since the resulting 4-*oxo*-retinoic acid is an important metabolite of retinoic acid (**1a**) and is biologically active,⁵⁾ this easy chemical conversion should be taken into account in experiments on and discussions of retinoic acid chemistry and biology.

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- 9) ¹H-NMR (400 MHz, ppm in CDCl₃) **5a** (methyl ester of **3a**, 6E, 12E): δ 6.73 (H₈), 6.44 (H₁₁), 6.37 (H₇), 6.34 (H₁₂), 6.04 (H₁₀), 5.77 (H₄), 3.69 (OCH₃), 3.10 (H₁₄), 2.13 (H₃), 1.92 (H₂₀), 1.91 (H₁₉), 1.89 (H₁₈), 1.51 (H₂), 1.29 (H_{16,17}).; **5c** (methyl ester of **3c**, 6Z, 12E): δ 6.64 (H₈), 6.44 (H₁₁), 6.37 (H₇), 6.29 (H₁₂), 6.02 (H₁₀), 5.62 (H₄), 3.69 (OCH₃), 3.09 (H₁₄), 2.13 (H₃), 2.07 (H₈), 1.92 (H₂₀), 1.91 (H₁₉), 1.51 (H₂), 1.12 (H_{16,17}).
- 10) Argon was deoxygenated through basic pyrogallol solution and then concentrated sulfuric acid. Some experiments were performed under freeze-degassed conditions.
- 11) Methyl 4-*oxo*-retinoates can be formed from **2a** by treatment with manganese dioxide,^{a)} or photochemically by iodine.^{b)} a) A. B. Barua, M. C. Ghosh, *Tetrahedron Lett.*, 1823 (1972).; M. S. S. Rao, J. John, H. R. Cama, *Int.J.Vitam.Nutr.Res.*, **368**, 42 (1972).; b) R. M. McKenzie, D. M. Hellwege, M. L. McGregor, E. C. Nelson, *Lipids*, **14**, 714 (1979).
- 12) Compound **4** was formed in 12 % yield when **2a** was kept in methanolic TFA (60 eq)-LiCl (30 eq) for 10 days at 23°C. However, the reaction afforded various products, such as methyl 4-methoxyretinoates (**6**, 26 %) and methyl 5-acetyl-4,18-*dinor*-retinoates (**7**, 14 %). The free acid of **7** is an active retinoid.

**6****7**

- 13) Oxidation of β -ionone to 4-alkoxy- β -ionone by electron-transfer reaction was reported. V. Calo, L. Lopez, L. Troisi, *J. Chem. Soc., Chem. Commun.*, 25 (1989).

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